REMARKS

CLAIM AMENDMENTS

Claim 1 has been amended to include the step "correlating the measured fluorescence polarization to protease activity." No new matter has been added. The claim as amended is supported by the disclosure, for example, at page 5, lines 3-5 of the specification.

Claims 3, 4, 8 and 9 have been amended to include proper Markush language. Claim 10 has been amended to depend from claim 2 as described below.

Claim 11 has been amended to correct obvious typographical errors and to include proper Markush language.

Claim 16 has been added as a new independent claim. The claim incorporates each of the limitations of original claim 10 as depending from original claim 6. No new matter has been added. The new claim is supported, for example, by claims 1, 6 and 10 as originally filed.

Upon entry of this amendment, claims 1-11 and 16 will be pending in the application.

REJECTIONS UNDER 35 U.S.C. § 112

1. CLAIM 10

Claim 10 stands rejected under 35 U.S.C. § 112 as being indefinite for failing to provide sufficient antecedent basis for the limitation "Abu." This rejection is respectfully traversed. As suggested by the Office, Applicants have amended claim 10 to be dependent from claim 2 rather than claim 3. Claim 2 provides clear antecedent basis for the limitation "Abu" as 2-aminobuteric acid is an aminoalkylcarboxylic acid. Withdrawal of the rejection is respectfully requested.

2. CLAIMS 1 AND 11

Claims 1 and 11 stand rejected under 35 U.S.C. § 112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. This rejection is respectfully traversed.

Applicants respectfully submit that originally filed claims 1 and 11 are sufficiently definite under 35 U.S.C. § 112, second paragraph for the reasons previously made of record. However, in order to further prosecution of the application, Applicants have amended claim 1 to

include a further method step "correlating measured fluorescence polarization to protease activity."

The Examiner has argued that the method of the present invention "does not have a resolution step which indicates what change in fluorescence activity will be indicative of being a protease inhibitor." Claims 1 and 11 are alternative methods for "measuring the activity of a protease" and "identifying compounds that inhibit a protease" respectively. Applicants respectfully submit that the critical step of the instant methods involves measuring the fluorescence polarization of the mixture. As amended, claim 1 comprises a final step of "correlating measured fluorescence polarization to protease activity" and claim 11 comprises a final step of "calculating the amount of protease inhibition." Such methods are sufficiently taught in Applicant's disclosure, for example, at page 8, line 31 to page 10, line 5 where Applicants teach calculations for correlating measured fluorescence polarization to protease activity and, for example, at page 10, line 7 to page 11, line 16 where Applicants describe a method for calculating the amount of protease inhibition from measured fluorescence polarization. Accordingly, it is respectfully submitted that claims 1 and 11 clearly set forth adequate method steps which are sufficiently definite such that one skilled in the art could practice the present invention. Withdrawal of the rejection under 35 U.S.C. §112, second paragraph is respectfully requested.

REJECTION UNDER 35 U.S.C. § 103

Claims 1-9 and 11 stand rejected under 35 U.S.C. § 103(a) as being obvious over Heath et al. (U.S. Pat. No. 5,235,039), Bromberg (U.S. Patent No. 4,203,670) and Maeda (Analytical Biochemistry, 1979) in view of Welch et al. (PNAS 1991) or Blakeslee et al. (J. of Immunological Methods, 1976). This rejection is respectfully traversed.

As acknowledged by the Office, the present invention provides a one-step assay for the determination of protease activity, particularly the activity of herpes virus proteases and human immunodeficiency virus proteases. As defined in claim 1, the method comprises a) incubating a mixture of a protease and a substrate capable of being bound to an anchor and having a fluorescent radical attached to the substrate; b) binding the substrate to the anchor; c) measuring the fluorescence polarization of the mixture; and d) correlating the measured fluorescence

polarization to protease activity. As described at page 5, lines 9-18 of the specification, the method of the present invention "has the advantage of being a solution phase determination of enzyme activity and requires no further manipulations other than addition of reagents at the appropriate times." Accordingly, the present method "is appropriate for adaptation in a high-throughput or semi-automated assay, and especially for a natural products screen since the polarization signal is derived from the ratio of fluorescence intensities and is less sensitive to contributions from background fluorescence. One can thus determine protease activity in the presence of a high fluorescence background."

To establish a *prima facie* case of obviousness, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to combine the teachings of prior art references. Further, the references, when combined, must teach all of the claim limitations. Finally, the prior art or knowledge generally available in the art must provide a reasonable expectation of success. See MPEP 2143.

1. There is no motivation, either in the references or the general knowledge of the art, to combine reference teachings.

The principal reference, Heath et al., describes a multi-step process for measuring protease activity using a substrate having a resin-binding compound and a fluorescent entity on opposite sides of a cleavage site. The method comprises a) incubating a proenzyme, the substrate and an activator; b) transferring the incubation solution to a multi-well plate having an upper and lower chamber separated by a porous membrane wherein the resin-binding compound is irreversibly bound to resin beads in one side of the chamber, (c) filtering and washing each of the two-chambered wells; and (d) analyzing the fluorescent emission of each well of the plate. The method described in Heath et al. is distinguishable from the method of the present invention in that Heath et al. describe a multi-step process wherein the accuracy of the measurement of polarization is a result of isolating the fluorescent entity from solution. Nothing in the reference remotely teaches or suggests a single step assay capable of measuring protease activity in solution which requires no further manipulations other than the addition of reagents at the appropriate times. Further, the method described by Heath et al. utilizes an entirely different method of fluorescent detection which does not lend itself for adaptation as a high-throughput or

semi-automated assay, and is not amenable for screening natural products having high a high fluorescence background. Accordingly, it is respectfully submitted that one skilled in the art reading the disclosure of Heath et al. would not be led to practice the methods of the present invention.

The second cited reference, Bromberg, U.S. Patent No. 4,203,670, describes an improved method for measuring the magnitude of the polarization of fluorescence of solutions. However, nothing in the reference would motivate one to use fluorescence polarization with the substrates described in Heath et al. In particular, the teachings of Bromberg et al. are entirely devoid of any mention of measuring fluorescence polarization in a solution comprising a protease or a any substrate capable of being bound to an anchor. Further, the reference fails to provide any teaching for determining protease activity or inhibition from measured fluorescence polarization. Thus, one skilled in the art would not be motivated to combine the teachings of Bromberg with Heath et al.

The third cited reference, Maeda, Analytical Biochem. 1979, describes the use of fluorescence polarization to assay proteolytic enzymes using fluorescein isothiocyanate-conjugated proteins as substrates. However, nothing in the reference would motivate one skilled in the art to combine the teachings of Maeda with that of either Heath et al. or Bromberg. In particular, Maeda does not describe the use of fluorescence polarization to measure protease activity with a peptidic substrate. Further, nothing in the reference describes binding the substrate to an anchor. Accordingly, one skilled in the art reading Heath et al., would not employ the methods of Maeda for measuring protease activity of a peptidic substrate. For example, as described at Column 1, lines 39-57 of Heath et al., peptidic substrates requiring recognition sites at each side of the cleavage site would not have a sufficient fluorescence differential to measure the extent of cleavage if used in the methods of Maeda without separating the fluorescent entity from solution. Accordingly, one skilled in the art would not be motivated to use the methods of Maeda with the substrates described in Heath et al.

Further, it is respectfully submitted that the deficiencies of the cited references cannot be overcome by resort to the teachings of Welch et al. or Blakeslee. Welch et al. describe a non-analogous protein screening assay for herpes virus proteinase using gel electrophoresis. Blakeslee merely describes DTAF and FTIC as alternative fluorescent markers. Thus, neither

Welch et al. nor Blakeslee provides any further teaching of relevance to the combination of cited references with respect to the methods of the present invention.

Because nothing in the cited references teaches or suggests the measurement of fluorescence polarization of a mixture comprising a protease and a peptidic substrate to determine protease activity or inhibition as required by the methods of the instant invention, it is respectfully submitted that that the present invention is patentable over the cited references Heath et al., Bromberg and Maeda in view of Welch et al. or Blakeslee. Reconsideration and withdrawal of the rejection under 35 U.S.C. §103(a) is respectfully requested.

2. The references, even if combined, do not teach all of the claim limitations.

Even if a motivation to combine the cited references could arguably be interpreted from the cited references or the general knowledge available to one skilled in the art, a prima facie case of obviousness is still lacking because the combined references do not teach all of the claim limitations. In particular, even if one practiced the fluorescence polarization methods of Bromberg or Maeda with the substrates of Heath et al., the combination of references is entirely devoid of any disclosure regarding "correlating measured fluorescence polarization to protease activity" as taught in the present invention and required by claim 1 or "calculating the amount of protease inhibition" as required by claim 11. Thus, it is respectfully submitted that any combination of the cited references does not teach all of the limitations of claims 1 and 11. Reconsideration and withdrawal of the rejection under 35 U.S.C. §103(a) is respectfully requested.

ALLOWABLE SUBJECT MATTER

The Examiner states that claim 10 would be allowable if rewritten in independent form. Accordingly, Applicants have re-written original claim 10 as new independent claim 16, which includes the limitations of the base claim and any intervening claims.

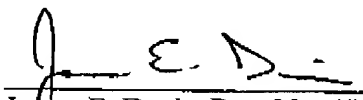
CONCLUSION

It is believed that all of the stated grounds of rejection have been properly traversed, accommodated, or rendered moot. Applicant therefore respectfully requests that the Examiner

reconsider and withdraw all presently outstanding rejections. It is believed that a full and complete response has been made to the outstanding Office Action, and as such, the present application is in condition for allowance. Thus, prompt and favorable consideration of this amendment is respectfully requested. If the Examiner believes that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at (314) 446-7683.

Applicants do not believe that any fee is required by the timely submission of this response. However, the Commissioner is hereby authorized to charge any required fees to Deposit Account No. 08-0750. Further, if there is any other fee deficiency or overpayment of any fees in connection with this patent application, the Commissioner is hereby authorized to charge such deficiency or credit such overpayment to Deposit Account No. 08-0750.

Respectfully submitted,



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Amendment C After Final Rejection (10 pages)
Facsimile Cover Sheet (1 page)